UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

AD-A226 562 PORT DOCUMENTATION PAGE

DTIC FILE COPY

1a. Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NMRI 90-64	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Naval Medical Research 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION Naval Medical Command
6c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055	7b. ADDRESS (City, State, and ZIP Code) Department of the Navy Washington, D.C. 20372-5120
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62233 / PROJECT NO. MM33C30.005 / TASK NO. 1051 / WORK UNIT ACCESSION NO DN249507

11. TITLE (Include Security Classification)
GM-CSF: A regulatory molecule for NK activity in the bone marrow

12. PERSONAL AUTHOR(S)
Davis TA, Monroy RL, Donahue RE, MacVittie TJ

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Book chapter	FROM _____ TO _____	1990	6

16. SUPPLEMENTARY NOTATION In: The Physiological and Pathological Effects of Cytokines. Edited by Charles A. Dinarello et al. New York: Wiley-Liss, 1990, pp.387-92.

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	GM-CSF; NK activity; hematopoietic growth factors, bone marrow, cytokines.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Phyllis Blum, Information Services Division	22b. TELEPHONE (Include Area Code) 202-295-2188 / 22c. OFFICE SYMBOL ISD/RSD/NMRI

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

The Physiological and Pathological
Effects of Cytokines, pages 387–392
Published 1990 by Wiley-Liss, Inc.

GM-CSF: A REGULATORY MOLECULE FOR NK ACTIVITY IN THE BONE MARROW.

Thomas A. Davis, Rodney L. Monroy, Robert E. Donahue, and Thomas J. MacVittie.

Immunobiology and Transplantation Department (T.A.D., R.L.M) Naval Medical Research Institute, Bethesda, MD. 20814. Genetic's Institute (R.E.D), Cambridge, MA. Experimental Hematology (T.J.M), Armed Forces Radiobiology Research Institute, Bethesda, MD. 20814

INTRODUCTION

The regulation of myeloid proliferation, differentiation and activation by granulocyte-macrophage colony stimulating factor (GM-CSF) has been recognized (~~Monroy et al., 1990~~). However, the role of GM-CSF in NK proliferation and activation is unclear even though NK cells have been shown to synthesize and secrete GM-CSF (~~Cuturi et al., 1989~~). The activation of peripheral blood NK cells (Dempsey et al., 1982) and their development from precursors in the bone marrow (BM) (~~Lotzova and Savary, 1987~~) are suggested to be regulated by multiple factors. We (~~Davis et al., 1990~~) have shown that in vivo administration of GM-CSF to normal monkeys resulted in a latent enhancement of peripheral blood NK activity, with no measurable change during the administration period. These suggested that GM-CSF treatment had an effect on NK cell development in the BM. To evaluate this possibility, we characterized the NK cell populations in the BM of normal primates during and after GM-CSF treatment. Our findings show a period of suppressed BM NK activity followed by the transitional appearance of a unique population of large lymphoid cells with an NK phenotype ($CD2^+$ $CD4^-$ $CD8^-$ $CD16^+$) and lytic activity.

Reprints (JS)

MATERIALS AND METHODS

Adult (male, 9.8 ± 1.6 kg) rhesus monkeys (Macaca mulatta) were used. Monkeys were injected (s.c., bid, 5 days) with GM-CSF (5×10^5 U/kg/day, Genetic's Institute, specific activity of 1×10^7 units/mg). Heparanized peripheral blood (3 ml) and iliac crest bone marrow (20 ml) were aseptically obtained from anesthesitized monkeys (ketamine hydrochloride, 10mg/kg, i.m.). All research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animal Resources", prepared by the Institute of Laboratory Animal Resources, National Research Council.

BM cells were separated by counterflow centrifugation-elutriation (CCE) as described elsewhere (Monroy et al., 1986). Briefly, Low density bone marrow cells (LDBMC, 78-450 x 10^6) isolated by isopycnic separation over Ficoll-Hypaque were injected into a Beckman JE-6B elutriation rotor system at a loading flow rate of 6ml/min and a rotor speed of 2000 rpm. Cell fractions were collected by stepwise increases in flow rate: Fraction I, 8.5 ml/min, Fraction II, 10.0 ml/min, and Fraction III (rotor-off fraction).

Natural killer cell activity was measured in a 4 hr ^{51}Cr release assay using a constant number (2.5×10^3) of K562 target cells and a variable number of mononuclear effector cells. Results expressed as percent specific release at an effector to target cell ratio (E:T) of 12:1.

RESULTS

Three distinct cell fractions were separated by CCE and morphologically characterized as (I) small lymphocytes, (II) intermediate-large lymphocytes, and (III) myeloid elements, containing 24%, 14% and 62% of the normal LDBMCs cells, respectively. The cellular composition and the number of cells recovered in fractions I and II did not change significantly over the longitudinal study. In contrast, the fraction III population was myeloid before and immediately following GM-CSF treatment but on days 21 and 35 a progressive increase in the number of lymphoid cells with a LGL morphology was detected.

The phenotype of each fractionated cell population is shown in Table 1. At day 7, a significant ($P \leq .01$) decrease (from 22% to 10%) in the percentage of CD2 positive cells was measured in LDBMCs due to an increase in myeloid

elements as a result of proliferation in response to GM-CSF. By day 35, 65% of the LDBMCs expressed a $CD2^+$ phenotype. In comparison to changes in the LDBMC population, no significant changes in the relative distribution of $CD4^+$, $CD8^+$ or $CD16^+$ were measured in fractions I and II, however an increase in $CD2^+$ cells as detected in both fractions on day 35. On days 21 and 35, the increase in frequency of lymphoid cells in fraction III with a large granular lymphoid (LGL) morphology was paralleled by a significant increase in the percentage of cells expressing a NK cell marker (CD16) and a T-cell marker (CD2) However, these cells did not express either CD4 or CD8 surface markers.

Table 1. Expression of cell surface antigens on LDBMCs and CCE separated bone marrow cells from monkeys treated with GM-CSF.

		Percent Positive Cells[a]			
Fraction	Day	CD2	CD4	CD8	CD16
LDBMCs	0	22 ± 3	6 ± 1	16 ± 2	3 ± 2
	7	10 ± 2	3 ± 1	6 ± 1	7 ± 6
	21	32 ± 6	9 ± 3	13 ± 4	4 ± 2
	35	65 ± 18	13 ± 1	29 ± 11	7 ± 2
Fraction I	0	58 ± 3	17 ± 2	44 ± 4	10 ± 4
	7	80 ± 4	22 ± 3	55 ± 3	3 ± 3
	21	57 ± 10	15 ± 1	45 ± 5	7 ± 4
	35	78 ± 13	22 ± 4	56 ± 9	7 ± 3
Fraction II	0	62 ± 12	21 ± 2	38 ± 7	12 ± 7
	7	67 ± 18	23 ± 8	41 ± 11	11 ± 9
	21	84 ± 6	25 ± 4	53 ± 7	10 ± 7
	35	79 ± 8	28 ± 2	49 ± 4	6 ± 4
Fraction III	0	1.2 ± 1	0.4 ± 1	0.3 ± 1	1.5 ± 1
	7	7 ± 5	1.6 ± 2	2.1 ± 2	2.2 ± 1
	21	8 ± 2	1.7 ± 1	2.0 ± 1	8.7 ± 3
	35	53 ± 9	3.9 ± 2	2.5 ± 1	15 ± 5

a. Separated cells were stained with FITC conjugated monoclonal antibodies, and percent positive cells determined by flow cytometry. Results expressed as the mean percent positive cells ± 1SD from 3 monkeys.

Table 2. Characterization of NK activity in LDBMCs and CCE separated bone marrow following GM-CSF administration.

Fraction	Day	NK activity[a] Prior Culture	Post Culture[b]
LDBMCs	0	53 ± 12	NT[c]
	7	27 ± 15	NT
	14	11 ± 8	NT
	21	29 ± 16	NT
	35	34 ± 7	NT
Fraction I	0	48 ± 12	68 ± 11
	7	20 ± 16	49 ± 7
	14	11 ± 4	NT
	21	27 ± 12	68 ± 10
	35	26 ± 12	69 ± 4
Fraction II	0	61 ± 7.5	82 ± 6
	7	39 ± 14	68 ± 13
	14	25 ± 5	NT
	21	47 ± 14	77 ± 3
	35	36 ± 5	72 ± 4
Fraction III	0	8 ± 8	12 ± 5
	7	10 ± 5	21 ± 11
	14	5 ± 7	NT
	21	20 ± 4	50 ± 16
	35	31 ± 3	32 ± 7

a. NK activity against K562 target cells in a 4-hr ^{51}Cr release assay. Results expressed as mean percent specific release + 1SD (n=3) at an E:T ratio of 12:1.
b. Bone marrow cells were cultured for 5 days with 20 units/ml of rIL-2.
c. Not tested (NT).

NK cell activities of the isolated BM cell populations are presented in Table 2. LDBMCs from monkeys prior to treatment contained significant NK activity, with almost all of the activity recovered in fraction I (~55%) and II (~45%) cells. NK activity in LDBMCs, fraction I and fraction II was reduced to 20-40% of normal on days 7 and 14, with no change in fraction III NK activity. Normal NK activity in these cell populations did not return until after 35 days. Significant NK activity was detected in fraction III cells

on days 21 and 35, and this increase of activity occurred when there was a high frequency of LGL and $CD2^+$ cells.

The suppressed NK activity in fractions I and II was abrogated by culture with rIL-2 (20 U/ml) for 5 days. The cytolytic capacity of fraction III cells was also significantly enhanced at day 21 by culturing in the presence of IL-2.

Unlike the bone marrow, no significant changes in peripheral blood activity was measured thru day 21, and in two monkeys peripheral blood NK activity was slightly elevated on days 28-35, returning to normal by day 42.

SUMMARY

We have demonstrated that GM-CSF administration to normal monkeys resulted in significant changes in NK cell activity within various bone marrow cell populations over a 5 week period. NK cell activity in the lymphoid fractions containing both small and large bone marrow NK cells was decreased for 2 weeks following GM-CSF treatment, returning to normal levels over the next 5 weeks. In addition, the recovery of the NK activity was accompanied by the appearance of large granular lymphocytes with a unique phenotype ($CD2^+$ $CD4^-$ $CD8^-$ $CD16^+$). Although the mechanism of action of GM-CSF on NK cell activity in the marrow is unclear, the results from these studies suggest that functional NK cells within the BM are decreased with GM-CSF administration and that this effect may be at the progenitor/precursor stage. Thus, we have identified that GM-CSF in vivo can affect not only NK cells in the peripheral blood but also the generation of functional NK cells in the BM. Furthermore, that this effect was detected over a prolonged period which reflected NK cell regeneration in the bone marrow.

ACKNOWLEDGMENTS

Views presented in this paper are those of the authors; no endorsement by the Department of Navy or the Defense Nuclear Agency has been given or should be inferred. This work was supported by the Naval Medical Research and Development Command, Research Task No.MM 33C30.1005 and the Defense Nuclear Agency Work Unit No.B2082. This work was done in partial fulfillment of the requirements for the degree of the Doctor of Philosophy (T.A.D) from George Washington University School of Arts and Sciences.

REFERENCES

Clark SC (1988). Biological activities of human granulocyte-macrophage colony-stimulating factor (1988). Int J Cell Cloning 6:365-377.

Cuturi MC, Anegon I, Sherman F, Loudon R, Clark SC, Perussia B, Trinchieri G, (1989). Production of hematopoietic colony-stimulating factors by human natural killer cells. J Exp Med 169:569-583.

Davis TA, Monroy RL, Skelly RR, Donahue RE, MacVittie TJ (1990). Differential augmentation of in vivo natural killer cytotoxicity in normal primates with recombinant human interleukin-1 and granulocyte-macrophage colony stimulating factor. J Clin Exp Immunol (in press).

Dempsey, RA, Dinarello CA, Mier JW, Rosenwasser LJ, Allegretta M, Brown TE, Parkinson DR (1982). The differential effects of human leukocytic pyrogen/lymphocyte activating factor, T cell growth factor and interferon on human natural killer activity. J Immunol 129:2504-2511.

Lotzova E, Savary CA (1987). Generation of NK cell activity from human bone marrow. J Immunol 139:279-284.

Monroy RL, MacVittie TJ, Darden JH, Schwartz GN, Patchen ML (1986). The rhesus monkey: a primate model for hemopoietic stem cell studies. Exp Hematol 14:904-911.

Monroy RL, Skelly RR, MacVittie TJ, Davis TA, Sauber JJ, Clark SC, Donahue RE (1987). The effects of recombinant GM-CSF on the recovery of monkeys transplanted with autologous bone marrow. Blood 70:1696-1699.

Monroy RL, Davis TA, MacVittie TJ (1990). Granulocyte-macrophage colony stimulating factor: more than a hemopoietin. J Clin Immunol Immunopath (in press)